

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Steady-state levels of RNA binding proteins are lower in skeletal muscle as compared to brain. A) Tissue lysates obtained from brain, kidney, liver and skeletal muscles were resolved by SDS-PAGE and immunoblotted for PABPN1 and MATR3. Steady state levels of PABPN1 are strikingly lower in skeletal muscle as compared to other tissues. Ponceau S stain and HSP90 immunoblot were used as loading controls. B) Mass spectrometric analysis of tissue lysate was used to compare the levels of RNA-binding proteins between murine brain and muscle (47). The levels of many RNA-binding proteins are lower in muscle when compared to brain as evident from the markedly lower Muscle to Brain LFQ ratio.

Supplementary Figure 2. PABPN1 immunoprecipitates with MATR3 primary myoblast lysate. Immunoprecipitations were performed from primary myoblast lysate using control IgG, a MATR3-specific antibody or a PABPN1-specific antibody. The input and bound samples were resolved by SDS-PAGE and immunoblotted for PABPN1, PABPC1 and MATR3. MATR3 and PABPN1 successfully immunoprecipitate each other and PABPC1 from myoblast lysate.

Supplementary Figure 3. PABPN1 and MATR3 interact in C2C12 myoblasts. PABPN1 was immunoprecipitated from proliferating (Pro) C2C12 myoblasts or C2C12 myoblasts grown in differentiation medium for 2 days (Diff 2) or 5 days (Diff 5) using a PABPN1 antibody or control IgG. Immunoblotting for PABPN1 show that a comparable amount of PABPN1 is immunoprecipitated under all three conditions. MATR3 co-immunoprecipitates with PABPN1 in lysates obtained from differentiating C2C12 myoblasts (Diff. 2 and Diff. 5).

Supplementary Figure 4. The PABPN1-MATR3 interaction is conserved in brain.

Immunoprecipitations were performed from murine brain lysate using either control IgG or a PABPN1-specific antibody. The input and bound samples were resolved by SDS-PAGE and immunoblotted for PABPN1, TDP43 and MATR3. PABPN1 co-immunoprecipitates both TDP43 and MATR3 from brain lysate.

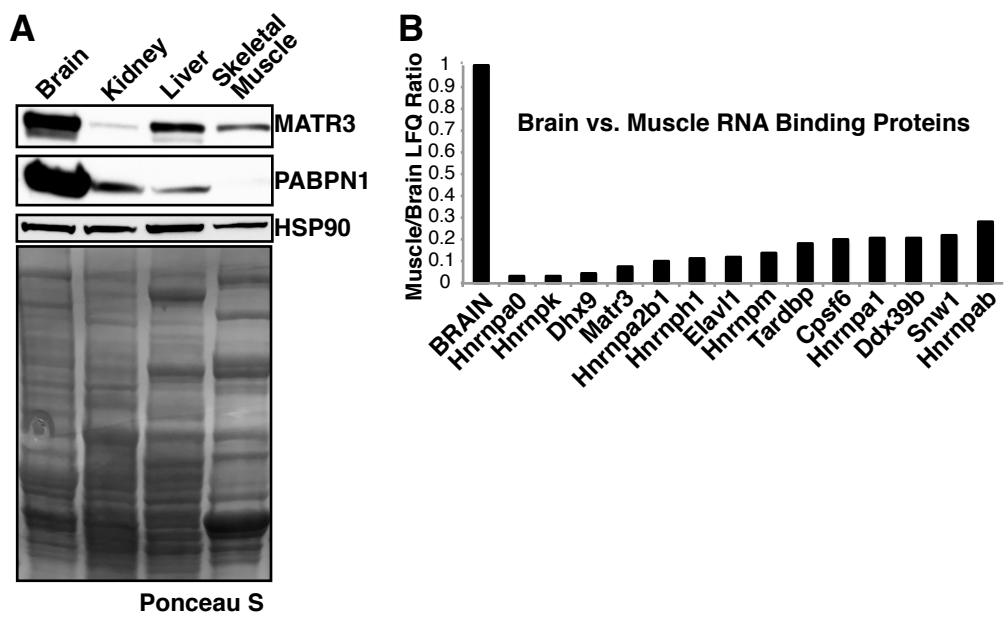
Supplementary Figure 5. A) Primary myoblasts were transfected with control siRNA or siRNA targeting PABPN1 or MATR3. qRT-PCR with primers detecting the CDS (Total) and the long 3' UTR (Distal UTR) was utilized to quantify levels of *Tmod1*, *Timp2*, *Psme3* and *Vldlr* RNAs. The levels of the transcripts are presented relative to control siRNA normalized to *Hprt*. The experiment was carried out at least five independent times. Error bars represent standard error of the mean (* p<0.05). B) qRT-PCR was performed to assess the levels of *Mat2a* transcript containing the retained intron (*Mat2a-RI*) and total *Mat2a* transcript in myoblasts depleted of PABPN1 and MATR3 as compared to control myoblasts. The levels of the transcripts are presented relative to control siRNA normalized to *Hprt*. The experiment was carried out seven independent times. Error bars represent standard error of the mean (* p<0.05).

Supplementary Figure 6. Depletion of PABPN1 and MATR3 stabilizes *Neat1* RNA in primary myoblasts. The half-life of *Neat1* was determined in control primary myoblasts or primary myoblasts transfected with siRNA targeting PABPN1 or MATR3. Myoblasts were lysed 0.5, 1, 2 and 4 hours after transcriptional arrest was initiated by treatment with actinomycin D. qRT-PCR was used to determine the amount of *Neat1* remaining at the various time points normalized to *Gapdh* transcript levels relative to 0.5 hours. Depletion of PABPN1 or MATR3 significantly stabilizes *Neat1* RNA when compared to control myoblasts. The error bars represent SEM from 4 biological replicates. * p<0.05.

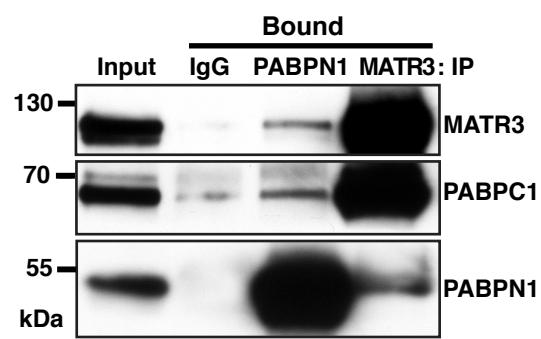
Supplementary Figure 7. Sequence analysis of cDNA clones from *Ctn* RNA demonstrates a *Neat1*-dependent increase in A to I editing in myoblasts depleted of PABPN1 or MATR3. RNA obtained from control myoblasts or myoblasts depleted of PABPN1 or MATR3 was reverse transcribed to obtain cDNA, followed by PCR amplification, cloning and sequencing of a region of the 3' UTR of *Ctn* previously shown to be edited (68). For each siRNA treatment every line indicates the sequence of the *Ctn* 3' UTR obtained from an independent clone. Sequences shaded in yellow were positive for at least one editing event with each edited nucleotide being shown in red font. The unedited genomic sequence is shown highlighted in green for reference.

Supplementary Figure 8. *Neat1* RNA is efficiently depleted by siRNA treatment. qRT-PCR was used to assess steady state levels of *Neat1* RNA in primary myoblasts transfected with control siRNA or siRNA targeting PABPN1, MATR3 or *Neat1*. Transfection with siRNA targeting *Neat1* results in a decrease in *Neat1* levels in myoblasts. Depletion of PABPN1 or MATR3 results in an increase in *Neat1* levels and these increased levels are reduced by concomitant transfection of *Neat1* siRNA.

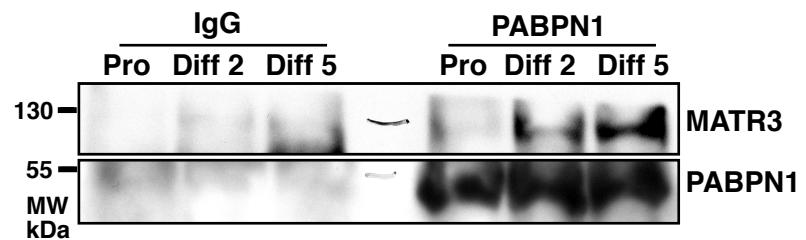
Supplementary Figure 9. Depletion of PABPN1 or MATR3 increase SFPQ foci in primary myoblasts. A) Primary myoblasts were transfected with control siRNA or siRNA targeting PABPN1 or MATR3, followed by staining for SFPQ (Red). DAPI (Blue) marks the nucleus. Cells depleted of PABPN1 or MATR3 show increased number of SFPQ foci as compared to control cells. Bar = 10 µm. B) Myoblasts were treated with Actinomycin D followed by staining for SFPQ. SFPQ relocates to perinucleolar caps (indicated by arrowhead) in cells treated with ActinomycinD. Bar = 10 µm.



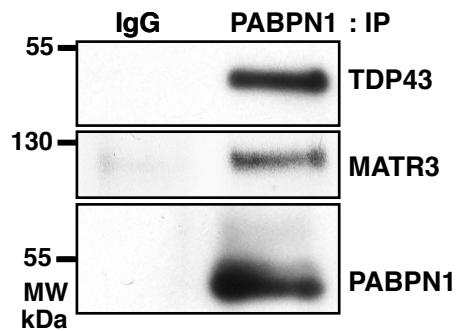
Supplementary Figure 1



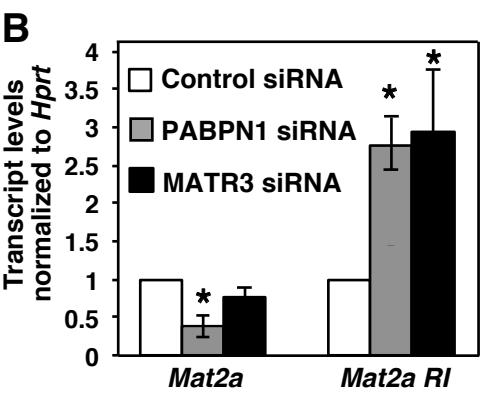
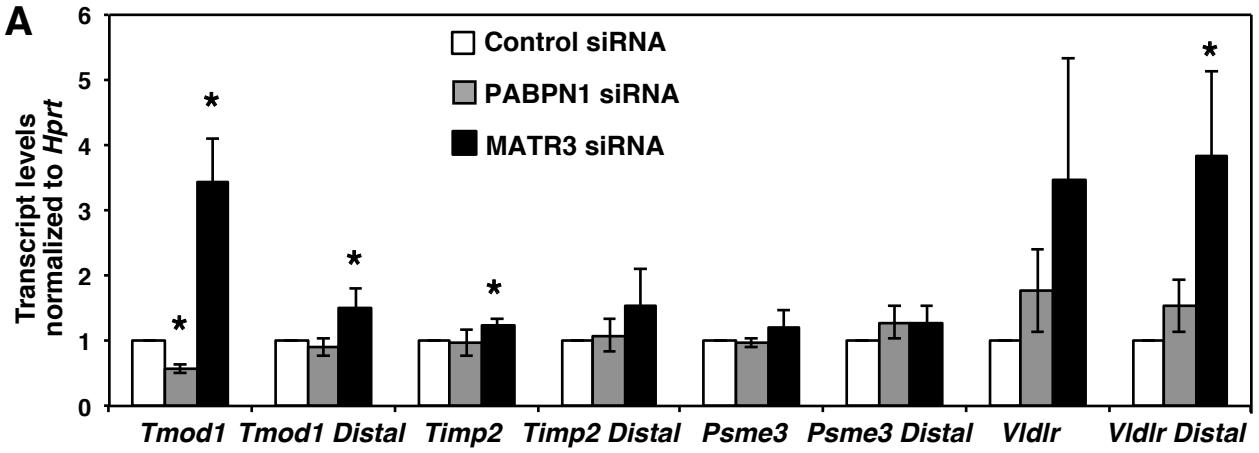
Supplementary Figure 2



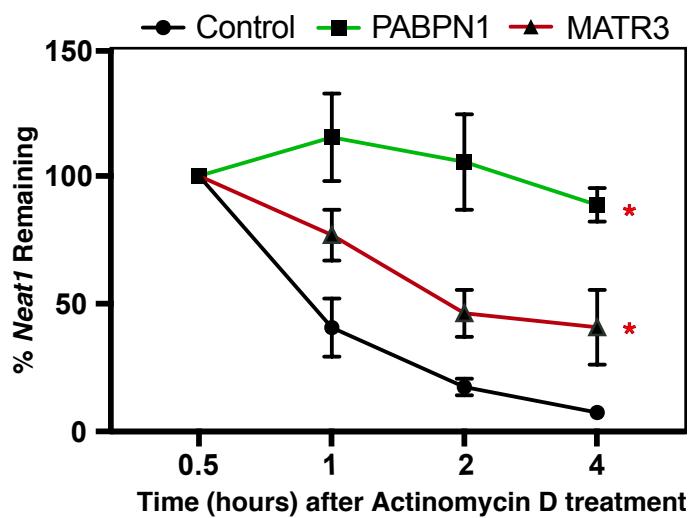
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6

CONTROL siRNA (3/18) 17%

CCCTGGA~~A~~CTCACTCTAAGACCA~~G~~ACTAACCTTGAACTCACAGAA~~T~~CCACCTGCCCTGCCCTCCC~~A~~GTAGCTGGATTAAAGGC~~G~~TGTGCCACCA

PABPN1 siRNA (9/17) 53%

CCCTGGA~~A~~CTCACTCT~~A~~TAGACC~~A~~GACT~~A~~ACCTTGA~~A~~CTCAC~~A~~GA~~A~~ATCC~~A~~CCTGCCTG~~C~~CCC~~A~~GTAGCTGGAATT~~A~~AAGGCGTGTGCCACCA

CCCTGGAGCTCACTATAGACCAGACTAACCTGAACTCACAGAAATCCACCTGCCTCTGCCTCCCAAGTAGCTAGAATTAAAGGCGTGTGCCACCA
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CCCTGGAACTCACTCTATAGACCAGACTAACCTGAACTCACAGAAATCCGCCTGCCTCTGCCTCCCAAGTAGCTGGAATTAAAGGCGTGTGCCACCA

MATR3 siRNA (9/22) 45%

CCCTGGAACTCACCTATAGACCAAGACTAACCTTGAACTCACAGAAATCCACCTGCCCTGCCCCAAGTAGCTGGAATTAAAGGCGTGTGCCACCA

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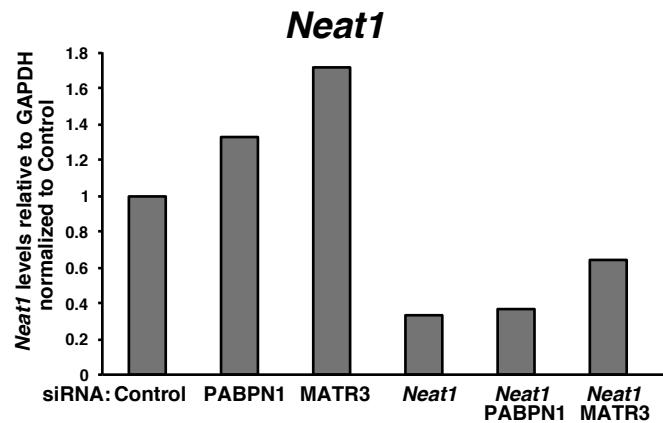
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CCCTGGAGCTCACTCTGTAGACCAGACTGACCTTGGACTCACGGAAGTCCGCCCTGCCTCCCAAGTAGCTGAAATTAAAGGCGTGTGCCACCA

Pabpn1+Neat1 siRNA (6/20) 30%

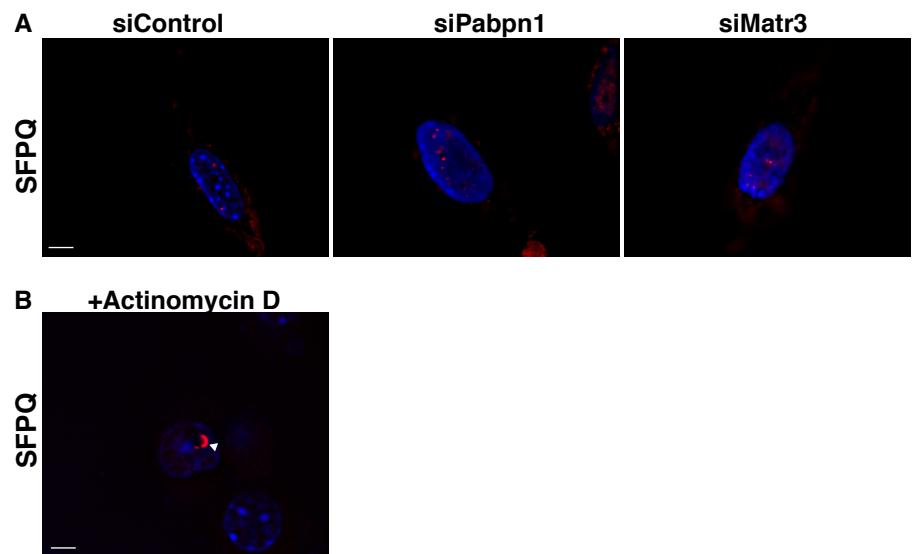
CCCTGGAACTCACTCT~~A~~TAGACC~~G~~ACT~~A~~CCCTGA~~A~~CTCAC~~AG~~~~AA~~ATCCACCTGCCTCTGCC~~CC~~~~A~~AGTAGCTGGATT~~AA~~AGGCGTGTGCCACCA

MATR3 +Neat1 siRNA (4/20) 20%

CCCTGGAACTCACTCTATAGACCAAGACTAACCTTGAACCTCACAGAAATCCACCTGCCCTGCCCTCCCAAGTAGCTGGAAITTAAGGCGTGTGCCACCA



Supplementary Figure 8



Supplementary Figure 9

Supplementary Table 1 (Oligonucleotides)

TCAGAAGAGTGAGAGAGAGCTAT	<i>Mat2a</i> mRNA F
CCATAGGCTGCAGTCCTC	<i>Mat2a</i> mRNA R
AAGTGGTTGCTCAAGGTT	<i>Mat2a</i> -RI F
CCTGGCTAACAAATACGAA	<i>Mat2a</i> -RI R
GAGCAGCAATGCAGAGACAC	<i>Psme3</i> Proximal F
CCAGTTTCCCATCACGATA	<i>Psme3</i> Proximal R
GCGAAGGTCAAACCCATAGA	<i>Psme3</i> Distal F
GGCAGGCTAATTGCAGAGAC	<i>Psme3</i> Distal R
GTCTAGCATGCGAAGGGAAAG	<i>Tmod1</i> Proximal F
GCACAAAGAACTGAGCCACA	<i>Tmod1</i> Proximal R
AGCTGCCAATTGATTCCAG	<i>Tmod1</i> Distal F
TCACCATTGCCTTTCTCC	<i>Tmod1</i> Distal R
CAGCGCTGAAGTCTCCTTTC	<i>Vldlr</i> Proximal F
GGGGCTCAAGGGTTACAGAT	<i>Vldlr</i> Proximal R
AGAATCTGCTGGACATTGG	<i>Vldlr</i> Distal F
TAAGACAGCGGTCTGGTGTG	<i>Vldlr</i> Distal R
TTTCTTGACATCGAGGACCC	<i>Timp2</i> Proximal F
TCCAGGAAGGGATGTCAAAG	<i>Timp2</i> Proximal R
ATGTGCGTGCTGGAATATGA	<i>Timp2</i> Distal F
CTGATACAGAGCATCAGGCG	<i>Timp2</i> Distal R
GATCGGGACCCAGTGACCTC	<i>Neat1</i> F
CAACAGCTTCCCCAACACCCAC	<i>Neat1</i> R
ATGTGTCCGTCGTGGAT	Mouse <i>Gapdh</i> f
CCTCAGTGTAGCCCAAGA	Mouse <i>Gapdh</i> r
TGGCTGTTCAACCGTACTTTC	<i>PAICS</i> F
CTTGCTTGATTTTC CTTCAGC	<i>PAICS</i> R

CACCAAGGAGGAAGAAGCAG	<i>NUP43</i> F
TCGGTTCCACAAACAAGACA	<i>NUP43</i> R
CCACTTGTCAGCTCATTC	Human <i>GAPDH</i> F
TTACTCCTGGAGGCCAT	Human <i>GAPDH</i> R
GCAAGAAAACCACAGAGGAGG	<i>Linc-MD1</i> F
GTGAAGTCCTGGAGTTGAG	<i>Linc-MD1</i> R
GGTCGAAGAGGACGACCAT	7SK-F
GCGCAGCTACTCGTATAACCC	7SK-R
PPM04481A (Qiagen)	<i>Myod1</i>
PPM04482A (Qiagen)	<i>Myogenin</i>
PPM05436C (Qiagen)	<i>Pitx2</i>
PPM05064E (Qiagen)	<i>Acta1</i>
GAAGGCCTACGAGCTGAGTG	<i>Mef2d</i> F
CTCTCGTGTGGCTCGTTGTA	<i>Mef2d</i> R
CATGTACGTTGAAGGCATGAGTTGGAAAC	<i>Malat</i> F
CTCTGCCTCCCAAGTGCTAGGAT	<i>Malat</i> R
Medium GC Negative Control siRNA	Control siRNA (Invitrogen)
UGUAGAAUCGAGAUCGGGAGCUGUU	PABPN1 Stealth™ siRNA (Invitrogen)
GAGAGUUCAUUUAUCCCAGAACGUAU	MATR3 Stealth™ siRNA (Invitrogen)
AUUCCAUGCUGCUAUCUAAAGGAA	Neat1 siRNA (IDT)
TTAAGCTGGTACCGAGCTGGATCCggccaccATGgaacagaagctgatcag agaggacctaATGTCCAAGTCATTCCAGCAGTC	Myc-MATR3-BamHI
CACTGTGCTGGATATCTGCAGAATTCTTAAGTTCTTCTTGCC TCCGTTTC	Myc-MATR3-EcoRI